Reference: *Biol. Bull.* **166**: 427–445. (April, 1984)

THE EFFECTS OF TEMPERATURE AND WATER AVAILABILITY ON ION AND ACID-BASE BALANCE IN HEMOLYMPH OF THE LAND HERMIT CRAB *COENOBITA CLYPEATUS*

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Abstract

Temperature acclimation (18–30°C) and dehydration (to 86% of initial mass) are two problems frequently encountered by the land hermit crab, *Coenobita clypeatus*. Their individual and combined effects on hemolymph ion and acid-base status were assessed. With free access to 10% SW, crabs maintained a constant degree of hydration, with hemolymph marginally hypo-osmotic to full strength SW. Increased acclimation temperature produced a reduction in pH, characteristic of ectotherms and consistent with maintenance of relative alkalinity which was accomplished by an elevation of CO_2 tension (P,CO_2). Hyperactivity resulted in some spillage of shell water and affected Cl^- balance. Under water deprivation, evaporative loss declined approximately exponentially and was negatively correlated with body mass. Hemolymph osmolality and electrolyte levels were significantly increased, ionic imbalance contributing largely to the hemolymph acidosis. Hemoconcentration was less marked when combined with temperature acclimation. Temperature-dependent pH regulation however in dehydrated crabs was accomplished as in hydrated crabs by ventilatory P,CO_2 control typical of air-breathers. The aquatic route of acid-base regulation (by ionic exchange) potentially afforded by the reservoir of water held in the molluscan shell was apparently not utilized.

Introduction

Adaptations allowing the transition of crustaceans onto land have evoked considerable study in an attempt to pinpoint criteria for terrestrial evolution. Morphological (Harms, 1932; Gray, 1957) and ecological surveys (Pearse, 1929; Gordon, 1956) have been supplemented more recently with investigations of physiological processes such as hydromineral regulation (Gross, 1963; de Wilde, 1973), nitrogen excretion (Gifford, 1968), respiratory gas exchange (Cameron and Mecklenburg, 1973; Cameron, 1975), and acid-base balance (McMahon and Burggren, 1981). Early advances were summarized by Bliss and Mantel (1968) while the most current information is the comprehensive physiological report of the Alpha Helix expedition to study the land crabs of the Palau Islands (see Cameron, 1981).

The two greatest problems facing terrestrial poikilotherms are no doubt temperature variation and dehydration (Edney, 1960). These two environmental features frequently

Received 12 July 1983; accepted 25 January 1984.

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Abbreviations: P,CO_2, partial pressure of CO_2; C,CO_2, total CO_2 content; pK'_1, apparent first dissociation constant of carbonic acid; α,CO_2, solubility coefficient of CO_2; $\Delta H^+m/c$, quantity of H^+ added by metabolic/respiratory acids; β, non-bicarbonate buffering capacity; ECFV, extracellular fluid volume; SID, strong ion difference.

interact in the natural terrestrial environment, yet this interdependence has rarely been considered in the study of physiological compensations. It was the purpose of the present study to examine the aspect of their combined action in the land hermit crab *Coenobita clypeatus,* which may owe its success in the terrestrial environment to the retention of the molluscan shell. This is generally water-filled and may confer resistance against desiccation (Reese, 1969; McMahon and Burggren, 1979).

Amongst the terrestrial decapods, studies have focused primarily on the Brachyura, especially *Cardisoma* and *Gecarcinus,* which are conspicuous in the supralittoral zone of the tropics and subtropics, and also on the monospecific anomuran genus *Birgus.* The terrestrial hermit crabs have received relatively little attention, perhaps because the adopted molluscan shell hinders physiological investigation. McMahon and Burggren (1979) studied respiratory gas exchange in *Coenobita* and investigated the individual effects of temperature acclimation (McMahon and Burggren, 1981) and dehydration (Burggren and McMahon, 1981) on acid-base balance.

Blood pH in ectothermic animals varies inversely with temperature and is actively regulated, according to the alphastat theory advanced by Reeves (1972, 1977). Water- and air-breathers, however, differ with respect to the mechanism of this regulation. Air-breathing vertebrates are thought to alter pH by adjusting the lung ventilation/CO_2 output ratio (*i.e.,* P,CO_2) on the principle of a closed, constant CO_2 system (Howell *et al.,* 1970; Jackson, 1971). Ventilatory control of blood P,CO_2 is not feasible in water-breathers since, in order to satisfy the O_2 requirement, ventilation is comparatively large and blood P,CO_2 is held correspondingly low since water-breathers hyperventilate with respect to CO_2 excretion requirements (Rahn, 1966). Instead, an open system usually operates to control the level of bicarbonate (McMahon *et al.,* 1978).

Since the bimodal breather *Coenobita* has access to both air and water from stores within the adopted shell, it could utilize either aerial (*i.e.,* control of P,CO_2) or aquatic (control of HCO_3^-) mechanisms of acid-base regulation. In an earlier study, McMahon and Burggren (1981) suggested that both mechanisms could operate in controlling extracellular pH. The purpose of the present study was to investigate whether the relative overall contribution of the two mechanisms reflects water availability.

Materials and Methods

Animal maintenance

The investigation was conducted on 26 individuals of *Coenobita clypeatus* (Herbst), identified using Bright (1966). For 2 weeks prior to experimentation they were housed in a $1.3 \times 0.4 \times 0.6$ m terrarium maintained at 30 ± 1°C (R.H. near to 40%). Crabs were fed liberally until the commencement of the experiment and provided with 10% sea water, the salinity they prefer (de Wilde, 1973).

Experimental design

The extremes of temperature studied were 18° and 30°C, which encompass the natural distribution of this species (Provenzano, 1959; de Wilde, 1973). Since ambient temperature varied, crabs were dehydrated to a constant water loss ($\simeq$14% of original body mass) sufficient to significantly elevate hemolymph osmolality (Burggren and McMahon, 1981). Total body mass (including shell water) was estimated as the difference between total mass (*i.e.,* shell plus animal) and the mass of the shell, since enforced shell evacuation is feared to affect the animals adversely. The mass of the inhabited *Livona* shells could be predicted confidently from external morphometric

measurements according to the procedure outlined by Wheatly (1984). Rehydration was subsequently examined. For comparison with the individual effect of temperature acclimation and desiccation, their combined action was investigated. Details of the experimental protocol are provided in Figure 1. Criteria considered in this design were; at least 4 days for temperature acclimation (Truchot, 1978) and rehydration, 2 days recovery from sampling prior to water deprivation, and 4 days between any two consecutive samples to avoid effects of repetitive sampling.

Hemolymph sampling and analytical procedures

Total mass (*i.e.*, animal plus shell) was recorded daily, always prior to sampling. 750–800 μl of prebranchial hemolymph was drawn into a 1 ml Hamilton gas-tight syringe with a 22-gauge needle inserted through the arthrodial membrane between the meropodite and coxopodite of the larger cheliped which was the only accessible site.

Osmolality and Cl^- concentration were determined immediately using a vapor pressure osmometer (Wescor 5100B) and digital chloridometer (Searle Buchler 4-2500) respectively. 100–200 μl of hemolymph were frozen rapidly and inorganic cations determined later by atomic absorption spectrophotometry (Jarrell-Ash 850). Mg^{2+} and Ca^{2+} were diluted 1 in 3000 and 500 respectively with 0.1% $LaCl_3 \cdot 6H_2O$, and K^+ and Na^+ were diluted 1 in 500 and 8000 respectively in 0.1% CsCl to suppress interferences in the air-acetylene flame.

pH was determined immediately on a 50 μl subsample using a liquid junction capillary electrode (Radiometer, G299A) connected to an acid-base analyzer (Radiometer PHM 71), calibrated against precision buffers and thermostatted to the appropriate temperature. Total CO_2 (C,CO_2) was measured on a 50 μl subsample using a Corning 965 CO_2 analyzer calibrated against standard bicarbonate solutions of 15 and 30 mmol l^{-1}. P,CO_2 was measured directly using a Radiometer electrode (E 5037-

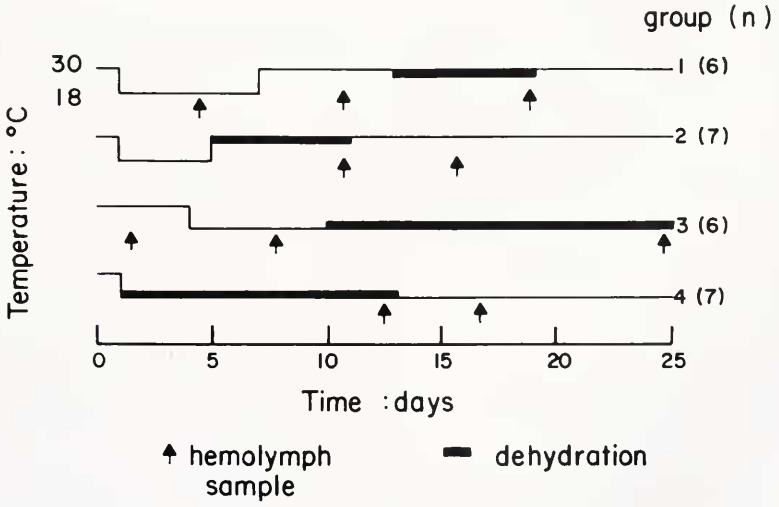

FIGURE 1. Diagrammatic representation of experimental protocol used to determine the responses of *Coenobita clypeatus* to a change in ambient temperature with and without access to free water, and progressive dehydration and rehydration at each acclimation temperature.

0) thermostatted to experimental temperature using a procedure modified from that of deFur *et al.* (1980). To minimize depletion of CO_2 from the sample and P,CO_2 gradients between hemolymph and electrolyte, two consecutive aliquots of $\simeq 100$ μl were flushed through the chamber at timed intervals, the syringe remaining in position to prevent introduction of air bubbles. At 18°C, intervals of two to three minutes were used while at 30°C, when equilibration was faster, two one minute periods were sufficient. The electrode was calibrated with millipore-filtered crustacean Ringer's solution equilibrated with humidified 1 and 2% CO_2 gas mixtures obtained from Wösthoff mixing pumps and P,CO_2 displayed on an acid-base analyzer (PHM 71) set to increased sensitivity ($\times 2$).

For the determination of lactate concentration 80 μl of hemolymph were precipitated in 400 μl of cold 12% perchloric acid, centrifuged, and the supernatant analyzed using a commercial kit (Sigma Technical Bulletin No. 826-UV). Serum protein concentration was determined by the Biuret method (Levin and Brauer, 1951) using a combined albumin/globulin standard (80 mg ml^{-1}).

Derived variables

(i) Molecular $CO_2 = \alpha CO_2 \cdot P,CO_2$—$\alpha CO_2$ is the solubility coefficient for CO_2 taken from Truchot (1976a) at the appropriate temperature and hemolymph ionic strength.

(ii) $[HCO_3^-] = C,CO_2 - \alpha CO_2 \cdot P,CO_2$.

(iii) $OH^-/H^+ = 10^{2(pH-pN)}$—an expression of "relative alkalinity" (see Howell *et al.*, 1973) where pN = 1/2 pKw (Austin and Cullen, 1925).

(iv) Δvariable/Δt—the coefficient of temperature variation.

(v) $\beta = \Delta[HCO_3^-]/\Delta pH$—the non-bicarbonate buffer capacity was calculated from the measured protein concentration using an equation derived for *Carcinus* by Truchot (1976b). McMahon and Burggren (1979) found that this equation was applicable to *Coenobita,* producing values similar to those measured in other land crabs (*e.g.,* Smatresk *et al.,* 1979).

(vi) pK'_1—Difficulties may arise from the use in analysis of apparent first dissociation constants of carbonic acid (pK'_1) determined in other species (see Wilkes *et al.,* 1980; Wheatly and McMahon, 1982). There was a considerable discrepancy between P,CO_2 calculated using pK'_1 for *Carcinus* (Truchot 1976a—see below) and the values presently measured. For this reason, to enable construction of a classical $[HCO_3^-]$ *versus* pH analysis, operational pK'_1 values were calculated for each treatment by substitution into the Henderson-Hasselbalch equation. If pK'_1 did not change significantly as a result of the experimental treatment values were combined for construction of P,CO_2 isopleths.

(vii) ΔH^+c and ΔH^+m—quantities of H^+ added by respiratory (c) and metabolic (m) acids respectively were estimated as in McDonald *et al.* (1979). Wherever the location of P,CO_2 isopleths were altered significantly as a result of the treatment, the point of intersection of the original P,CO_2 at the final location of the buffer line was considered. Paired observations were not available for groups 2 and 4 whose mean values were thus analyzed against the partner group (hence no variability).

(viii) $\Delta[HCO_3^-]$ and $\Delta[Ca^{2+}]$—changes in hemolymph $[HCO_3^-]$ and $[Ca^{2+}]$ in addition to those arising as a spurious consequence of hemoconcentration/dilution. In calculating the latter the assumption was made that changes in hemolymph osmolality were representative of changes in circulating volume.

Statistical treatment of data

Data are given as mean ± SEM (n) and Student's *t*-test performed on paired variates using $P < 0.05$ as the confidence level. Wherever values are presented as a percentile, statistical computations were performed on raw data. Linear regression (least squares method) was performed where appropriate.

RESULTS

Osmo- and ionoregulation

Temperature acclimation in hydrated crabs. Changes in mean body mass resulting from temperature acclimation and hemolymph sampling (groups 1 and 3) are given in Table I and compared with values for rehydrated crabs (groups 2 and 4). On the basis of previous work (see Weymouth *et al.*, 1944; Scholander *et al.*, 1953), errors introduced from weight loss due to metabolic carbon were considered negligible in the present study and changes in mass were assumed to represent body and/or shell water. Individual animals with free access to water maintained a relatively constant mass, with daily variation around the mean of 0.2–1.2 g (equivalent to 0.4–2.0%). Crabs acclimated to 30°C weighed significantly less (−2.5 g). Hemolymph sampling resulted in a loss of 3.6 g body water at 18°C and 1.0 g at 30°C. No overhydration occurred when crabs in groups 2 and 4 were rehydrated.

Corresponding hemolymph osmolality and ion levels are detailed in Table II. There were few significant differences between hydrated groups at the same temperature. $[Cl^-]$ in group 3 was higher at 18°C and lower at 30°C than group 1. $[Ca^{2+}]$ was significantly lower in rehydrated crabs at both 18°C and 30°C and $[Cl^-]$ was higher following rehydration at both temperatures by as much as 70 m-equiv l^{-1} at 18°C. Acclimation to a change in temperature produced a significant increase in

TABLE I

Mean and variation in mass of hydrated Coenobita clypeatus *acclimated to 18 or 30°C both prior to and subsequent to hemolymph sampling*

	18°C		30°C	
Group	Pre	Post	Pre	Post
Group 1 n = 6				
Mass $\bar{x}$ ± SEM	78.4 ± 5.0	74.7 ± 4.3	72.1 ± 4.0	71.9 ± 4.1
Variation $\bar{x}$ ± SEM	0.6 ± 0.1	0.3 ± 0.2	0.8 ± 0.1	0.8 ± 0.1
Group 2 n = 7				
Mass $\bar{x}$ ± SEM	75.2 ± 6.5		74.7 ± 7.1*	
Variation $\bar{x}$ ± SEM	0.5 ± 0.1		0.8 ± 0.2	
Group 3 n = 6				
Mass $\bar{x}$ ± SEM	59.5 ± 4.4	57.1 ± 5.0	59.2 ± 3.4	57.5 ± 4.5
Variation $\bar{x}$ ± SEM	0.4 ± 0.1	0.2 ± 0.1	0.8 ± 0.1	1.2 ± 0.5
Group 4 n = 7				
Mass $\bar{x}$ ± SEM	78.9 ± 9.4*		80.4 ± 10.0	
Variation $\bar{x}$ ± SEM	0.4 ± 0.1		—	

* Rehydrated animals. The units are g throughout.

TABLE II

Hemolymph inorganic ion concentrations and osmolality in hydrated and rehydrated hermit crabs at 18 and 30°C

		18°C						30°C					
		mOsm Kg^{-1} OSMOLAL	m-equiv l^{-1} $[Cl^-]$	$[Na^+]$	$[K^+]$	$[Mg^{2+}]$	$[Ca^{2+}]$	mOsm Kg^{-1} OSMOLAL	m-equiv l^{-1} $[Cl^-]$	$[Na^+]$	$[K^+]$	$[Mg^{2+}]$	$[Ca^{2+}]$
Group 1	$\bar{x}$	969	337	376	8	59	23	973	387*	375	9	54	28
(n = 6)	± SEM	±15	±11	±7	±1	±1	±2	±22	±13	±10	±1	±3	±1
								→					
2 Rehydrated								993	402**	378	10	49	21**
(n = 7)								±17	±8	±8	±0	±2	±1
3		941	383†	384	8*	60*	26	1005	352†	383	10	52	24
(n = 6)		±23	±6	±10	±1	±3	±1	← ±18	±7	±6	±1	±0	±1
4 Rehydrated		998	411**	367	7	59	19**						
(n = 6)		±21	±10	±18	±0	±5	±1						

The arrow indicates the direction in which the temperature change was effected. (Values are mean ± SEM.)

* Significant differences (paired) arising from a change in acclimation temperature of hydrated crabs.

** Significant differences (unpaired) resulting from a change in acclimation temperature of rehydrated crabs (compared with partner group control).

† Significant differences (unpaired) between groups of hydrated crabs at the same acclimation temperature.

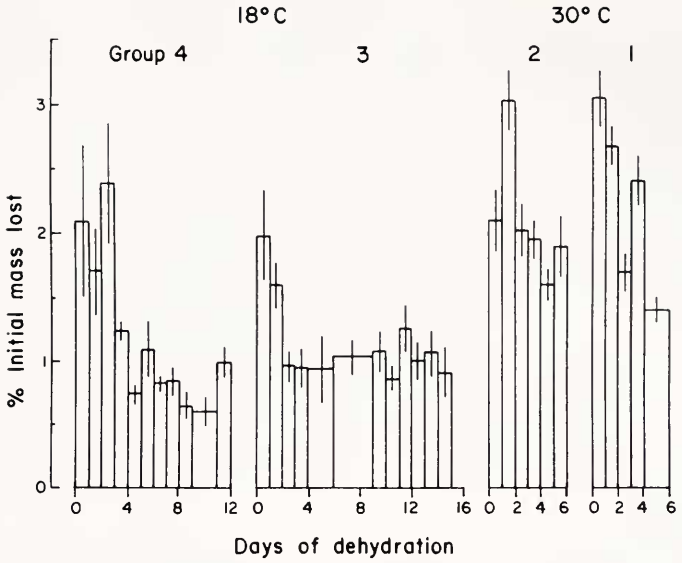

FIGURE 2. Rates of weight lost (presumably as water) with time during dehydration in *C. clypeatus* at 18 and 30°C. Mean ± SEM (n numbers as indicated in legend to Fig. 1).

[Cl^-] irrespective of the direction of temperature change and a reduction in ambient temperature also affected circulating [Mg^{2+}] and [K^+].

De- and rehydration at constant temperature. In each group mean weight loss declined exponentially with time (Fig. 2). Crabs temperature acclimated prior to dehydration (groups 1 and 3) exhibited the greatest loss on day 1. The settled rate of water loss at 30°C ($\simeq$1.5% initial mass · day^{-1}) was double that occurring at 18°C, and negatively correlated with body mass on a weight specific basis (Fig. 3).

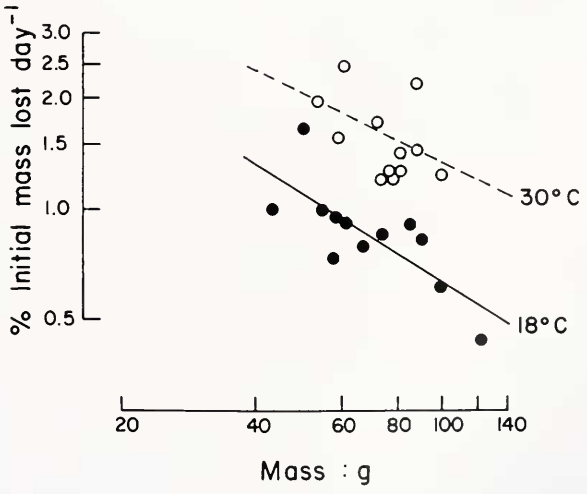

FIGURE 3. The relationship between settled weight specific water loss and weight in *C. clypeatus* at 18 (●) and 30°C (○). Regression equations (on $\log_e$ values): 18°C: Y = 3.18 − 0.79X (n = 12, r = 0.76); 30°C: Y = 3.18 − 0.63X (n = 12, r = 0.47).

The cumulative weight lost as water was comparable in each group. Mean weight loss suffered in treatments 1–4 were 12.6 ± 0.7, 12.6 ± 0.8, 15.9 ± 1.9, and 13.6 ± 1.5% initial mass occurring over approximately 6 or 14 days depending on acclimation temperature.

Osmolality and concentrations of all ions (except K^+) were increased as a result of dehydration (Fig. 4a *c.f.* Table II), percentage changes varying for different ions. Ion levels changed consistently at both acclimation temperatures except for Ca^{2+} which exhibited double the elevation at 18°C. These trends were essentially reversed on rehydration (Fig. 4b).

Temperature acclimation in dehydrating crabs. Crabs experiencing dehydration and temperature acclimation concurrently displayed maximum weight loss on day 2–3 (Fig. 2—groups 2 and 4) accompanied as before by significant increases in osmolality and inorganic ion levels (Fig. 5). The increases in osmolality and $[Ca^{2+}]$ were only one half the values reported above (*c.f.* Fig. 4a). The increase in $[Cl^-]$ was considerably in excess of osmolality and Na^+, but was roughly equivalent to the sum of the changes predicted in response to the individual environmental stimuli.

Acid-base balance

Temperature acclimation in hydrated crabs. The characteristic reduction in pH with an increase in acclimation temperature was accompanied by a threefold increase in P,CO_2, while C,CO_2 was unchanged (Table III). Upon lowering ambient temperature

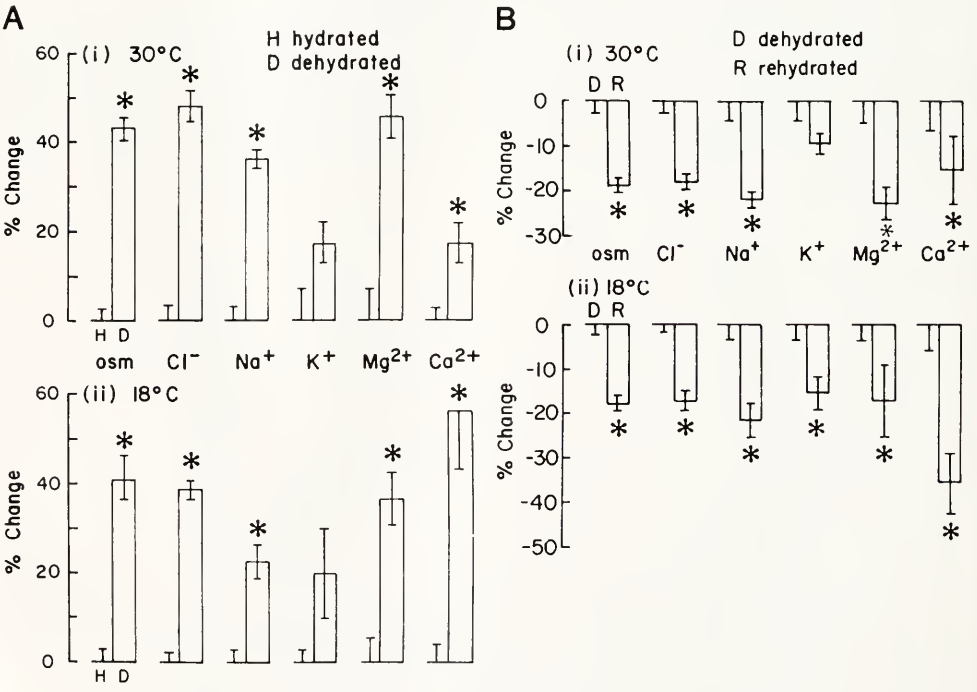

FIGURE 4. Percentile changes in hemolymph osmolality and inorganic ions during dehydration (a) and rehydration (b) at 30°C (i) and 18°C (ii). Asterisks denote significant differences (statistical tests performed on raw data). H, D, and R refer respectively to hydrated, dehydrated, and rehydrated states.

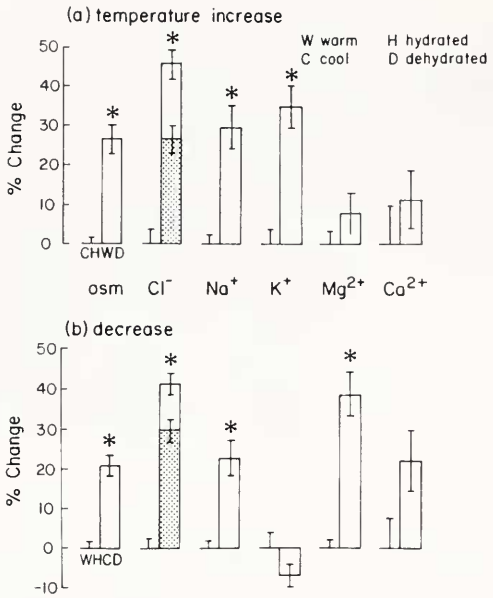

FIGURE 5. Percentile changes in hemolymph osmolality and inorganic ions during dehydration combined with an increase (a) or decrease (b) in ambient temperature. Statistical tests were performed on raw data. W, C, H and D refer respectively to warm, cool, hydrated, and dehydrated states. Stippled area indicates rise in $[Cl^-]$ attributable to temperature acclimation.

these trends were reversed; additionally C,CO_2, $[HCO_3^-]$ and lactate were significantly reduced. As expected, OH^-/H^+ was maintained constant ($\simeq$10). Coefficients of temperature variation were generally larger with an increase in temperature. Assessment of the origin of this acid-base disturbance on a $[HCO_3^-]$ *versus* pH diagram (Fig. 6) indicated that it originated predominantly from respiratory sources (see ΔH^+c—Table III) although metabolism contributed significantly especially when temperature was decreasing. In response to an increase in acclimation temperature $\Delta[Ca^{2+}]$ and $\Delta[HCO_3^-]$ increased in the ratio of 2:1 (Table III) but the reverse trend was not evident in the opposite direction of temperature change.

De- and rehydration at constant temperature. Corresponding variations in major determinants of acid-base balance on dehydration (Table IVa; Fig. 7) however, revealed that the accompanying acidosis was possibly attributable to ionic changes (OH^-/H^+ dropping to $\simeq$4). Moreover $\Delta[Ca^{2+}]$ and $\Delta[HCO_3^-]$ decreased, again in the ratio of 2:1. Converse trends of reduced magnitude were apparent on rehydration (Table IVb; Fig. 7). Significant changes in circulating lactate were only evident at 30°C where a reduction occurred upon dehydration.

Temperature acclimation in dehydrating crabs. In the absence of free water, an increase in temperature (Table Va; Fig. 8) resulted in a hemolymph acidosis attributable mainly to respiration, but less pronounced than the sum of the responses observed to the individual stresses. An additive response was apparent however, in the case of a reduction in acclimation temperature (Table Vb; Fig. 8). In summary, when plotted as a function of acclimation temperature (Fig. 9) it is apparent that the mechanism of pH regulation is the same in both hydrated and dehydrated crabs and is accomplished by an increase in P,CO_2 which is characteristic of air-breathing animals.

TABLE III

Variation in major determinants of extracellular acid-base state in hydrated Coenobita clypeatus *acclimated to 18 and 30°C*[1]

		a) Increase in acclimation temperature			b) Decrease in acclimation temperature		
		18°C			30°C		
Variable	Units	$\bar{x}$	SE(6)	$\Delta/\Delta t$	$\bar{x}$	SE(6)	$\Delta/\Delta t$
pH		7.613	±0.012	−0.019	7.449	±0.028	±0.014*
P,CO_2	torr	6.8	±1.0	+1.337	17.9	±1.5	−0.993*
C,CO_2	mmol l^{-1}	12.6	±1.8	+0.203	14.9	±0.9	−0.446*
HCO_3^-	mmol l^{-1}	12.3	±2.2	+0.160	14.2	±0.9	−0.417*
CO_2	mmol l^{-1}	0.33	±0.05	+0.041	0.65	±0.05	−0.030*
OH^-/H^+		8.6	±0.4	−0.064	11.6	±1.3	−0.159
Lactate	mmol l^{-1}	6.5	±0.4	−0.014	6.4	±0.7	−0.243*
				Δ			Δ
Protein	mg ml^{-1}	125.7	±6.7	−32.5*	113.8	±9.8	−14.7
β	mmol l^{-1} pH $unit^{-1}$	18.3	±0.9	−4.5*	16.6	±1.4	−2.0
pK'_1		6.06	±0.03	+0.08	6.11	±0.06	+0.01
ΔH^+m	m-equiv l^{-1}			+0.9 ± 1.4			−2.2 ± 1.0
ΔH^+c	m-equiv l^{-1}			+4.2 ± 0.6			−3.0 ± 0.7
$\Delta[Ca^{2+}]$	m-equiv l^{-1}			+4.8 ± 2.1			+2.2 ± 2.7
$\Delta[HCO_3^-]$	m-equiv l^{-1}			+2.5 ± 2.8			−3.7 ± 1.7

[1] The effect of a) increase and b) decrease in acclimation temperature. Asterisks beside coefficient of temperature variation denote that values changed significantly on temperature acclimation. Consult text for calculation of derived variables.

DISCUSSION

Temperature acclimation of hydrated crabs

The present investigation suggested that water economy was controlled to some extent in *C. clypeatus* (Table I). Crabs transferring to more capacious shells did not load extra water and there was no evidence of overhydration when water became available again after deprivation. The loss of ≃2.5 g of total mass on acclimation to 30°C probably represents water loss from the shell reservoir, arising unavoidably from heightened activity at increased temperature. The reduction in mass after sampling may be similarly attributed to shell water spillage since it was greater at 18°C.

With free access to 10% sea water in the present study *C. clypeatus* maintained the hemolymph slightly hypo-osmotic and hypoionic compared with full strength sea water (Table II—*c.f.* Gross, 1955; de Wilde, 1973). Thermal acclimation did not consistently affect these levels except for Cl^-. The increase following a rise in acclimation temperature may result from exchange for endogenous bicarbonate as a mechanism for regulating pH (see Randall and Cameron, 1973, and below). Less easily explained is the increase in Cl^- upon cooling which reaffirms our poor understanding of Cl^- regulation in the decapods (Gross, 1963; Burggren and McMahon, 1981). The significant change in Mg^{2+} and K^+ in group 3 presently cannot be explained.

Values reported for pH, P,CO_2, and $[HCO_3^-]$ in *C. clypeatus* (Table III) were generally similar to those of other air-breathing decapods at comparable temperatures (Cameron and Mecklenburg, 1973; Smatresk *et al.*, 1979; Wood and Randall, 1981).

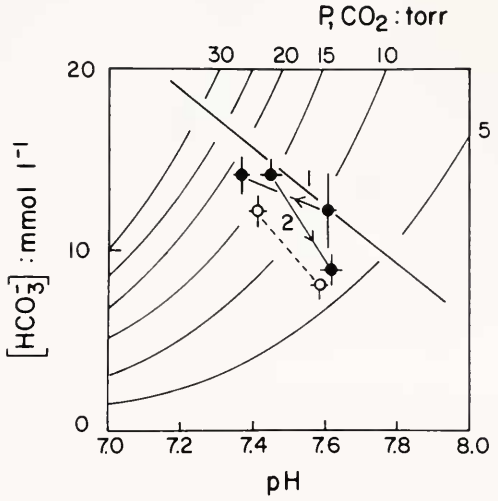

FIGURE 6. $[HCO_3^-]$ *versus* pH diagram indicating changes in acid-base status in prebranchial hemolymph of *Coenobita clypeatus* during temperature acclimation of hydrated animals. 1 and 2 indicate temperature increase and decrease respectively. Open symbols indicate acid-base status of rehydrated animals at corresponding acclimation temperatures. P,CO_2 isopleths were constructed using mean values of 6.102 for pK'_1 and 0.0425 mmole l^{-1} $torr^{-1}$ for α. For graphical purposes the average slope of the CO_2 buffer line is indicated.

An OH^-/H^+ ratio of $\simeq$9 is close to values reported by Truchot (1973) for the intertidal shore crab *Carcinus* ($\simeq$12). Since values for aquatic species are around 16–25 (Howell *et al.*, 1973) it would appear that relative alkalinity decreases with reliance on air-

TABLE IV

Variation in major determinants of extracellular acid-base balance in Coenobita clypeatus *as a result of (a) dehydration and (b) rehydration at 18 and 30°C*[1]

	(a) Dehydration		(b) Rehydration	
Variable	18°C Δ	30°C Δ	18°C Δ	30°C Δ
pH	−0.118*	−0.189*	+0.055	+0.055
P,CO_2	+3.4	−3.1	−2.0*	−4.5
C,CO_2	+5.8*	+1.2	−2.9*	−1.8
HCO_3^-	+4.5	+1.3	−2.8	−1.7
CO_2	+0.15	−0.15	−0.09*	−0.12
OH^-/H^+	−4.0*	−4.4*	+2.1	+2.3
Lactate	−1.6	−5.2*	+0.7	+1.6*
Protein	−16.8	−16.9	−7.7	−9.7
β	−2.3	−2.4	−1.1	−1.4
pK'_1	−0.08	−0.30*	+0.03	+0.05
ΔH^+m	+2.7 ± 0.2	+1.6 ± 0.2	−1.8 ± 1.3	−0.8 ± 0.8
ΔH^+c	+2.1 ± 0.5	−0.5 ± 0.6	−1.3 ± 0.4	−0.9 ± 0.2
$\Delta[Ca^{2+}]$	−8.0 ± 3.4	−18.1 ± 1.2	−1.3 ± 2.3	+5.9 ± 3.3
$\Delta[HCO_3^-]$	−2.9 ± 0.4	−9.8 ± 1.0	+0.9 ± 1.2	+3.8 ± 1.0

[1] Units and symbols—as for Table III.

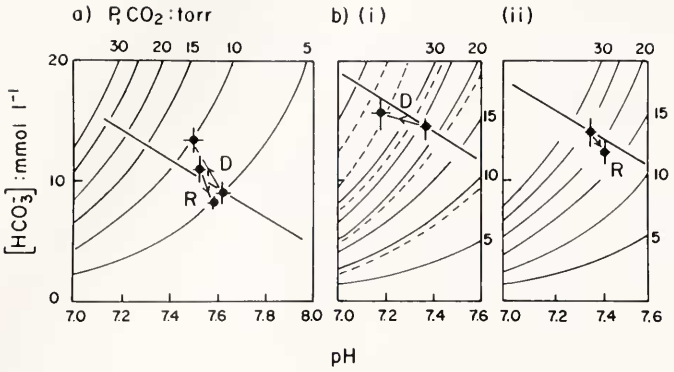

FIGURE 7. Changes in acid-base status in prebranchial hemolymph of *Coenobita clypeatus* during dehydration (D) to 86% of initial mass and subsequent rehydration (R) at 18°C (a) and 30°C [b (i) and (ii)]. At 18°C, P,CO_2 isopleths were constructed using mean values of 6.045 for pK'_1 and 0.048 mmole l^{-1} $torr^{-1}$ for αCO_2. In b (ii) mean values employed were 6.120 and 0.035 mmole l^{-1} $torr^{-1}$ respectively. At 30°C there were significant differences between operational pK'_1 for hydrated and dehydrated crabs. In b (i) solid isopleths correspond to hydrated crabs (pK'_1 = 6.135, α = 0.036 mmole l^{-1} $torr^{-1}$) and broken isopleths to dehydrated crabs (pK'_1 = 5.837 and α = 0.034 mmole l^{-1} $torr^{-1}$).

breathing as in ectothermic vertebrates (Howell *et al.*, 1970). A discrepancy between measured P,CO_2 values and those calculated taking pK'_1 from Truchot's (1976a) nomographs for *Carcinus* (interpolated as 6.06 and 5.99 respectively at 18 and 30°C in the hydrated state) probably relates to a higher value for β (see Methods) in the terrestrial species (*c.f.*, McMahon *et al.*, 1978; Smatresk *et al.*, 1979). This explains

TABLE V

Variation in major determinants of extracellular acid-base balance in Coenobita clypeatus *on dehydration combined with a) increase and b) decrease in acclimation temperature*[1]

	a) Increased acclimation temperature			b) Decreased acclimation temperature		
Variable	Δ		Δ/Δt	Δ		Δ/Δt
pH	−0.249*	(−0.429)	−0.020	+0.082*	(+0.053)	+0.007
P,CO_2	+15.9*	(+13.6)	+1.275	−10.8*	(−9.0)	−0.862
C,CO_2	+2.1	(+3.7)	+0.168	−3.5*	(+0.3)	−0.276
HCO_3^-	+1.6	(+3.3)	+0.130	−3.2	(+9.7)	−0.252
CO_2	+0.44*	(+0.36)	+0.035	−0.31*	(−0.23)	−0.025
OH^-/H^+	−1.0	(−5.2)	−0.080	−5.4*	(−6.0)	−0.432
Lactate	−4.4*	(−5.4)	−0.355	−2.2*	(−4.6)	−0.178
Protein	−29.3*			−21.7		
β	−4.1*			−3.0		
pK'_1	+0.04			−0.09		
ΔH^+m	−1.2	(+2.5)		−1.5	(+0.5)	
ΔH^+c	+2.9	(+3.7)		−1.6	(−0.9)	
$\Delta[Ca^{2+}]$	−8.9	(−13.3)		−4.9	(−5.8)	
$\Delta[HCO_3^-]$	−4.3	(−7.3)		−8.4	(−6.6)	

[1] Values in parentheses are those which can be predicted from addition of responses to individual stimuli. Units and symbols—as for Table III.

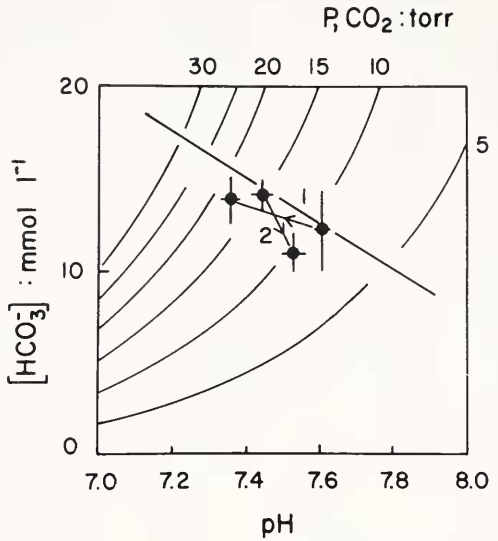

FIGURE 8. Changes in acid-base status in prebranchial hemolymph of *Coenobita clypeatus* on dehydration combined with acclimation to (1) increasing and (2) decreasing ambient temperatures. The mean values for pK'_1 and αCO_2 used in the construction of CO_2 isopleths were 6.087 and 0.0415 mmole l^{-1} $torr^{-1}$ respectively.

why P,CO_2 values presently measured were higher than those calculated using these constants in *C. brevimanus* in an earlier study (McMahon and Burggren, 1979). An alternative explanation could reside in their separate distribution in the natural environment, where *C. clypeatus* occurs further from the sea (Borrodaile, 1903; Bright, 1966). Resting lactate levels were higher than in aquatic species (*e.g.*, Booth *et al.*, 1982) and may be associated with sustained tonic activity in cheliped muscles associated with closure of the shell operculum.

The present data for temperature-dependent pH regulation in *Coenobita* (Table III; Fig. 6) produced $\Delta pH/\Delta t$ values characteristic of other ectotherms (Rahn, 1966). The similarity of these to $\Delta pK/\Delta t$ of various intracellular buffers is now thought to be more complex than originally envisioned in the "alphastat" theory advanced by Reeves (1972; see Cameron and Kormanik, 1982). Other authors (*e.g.*, Ackerman and White, 1980) have investigated the dependence of $\Delta pH/\Delta t$ on the direction of temperature change with similar results to the present study.

The most important single finding of the present investigation was that pH was controlled by the ventilatory regulation of P,CO_2, irrespective of the availability to the animal of free water and thus the provision of an aquatic route for HCO_3^- exchange (Fig. 9). Most air-breathing species do not have simultaneous contact with water. Therefore circulating $[HCO_3^-]$ is generally maintained during an increase in temperature whereas a decrease is observed in aquatic species (Cameron and Batterton, 1978; McMahon *et al.*, 1978). In animals where both mechanisms operate simultaneously (*e.g.*, the turtle—Howell *et al.*, 1970 or shore crab—Truchot, 1973), a decrease in $[HCO_3^-]$ generally accompanies a rise in P,CO_2.

The increase in $[HCO_3^-]$ in *Coenobita* was therefore largely unexpected (Fig. 9), although Heisler *et al.* (1980) made a similar observation in dogfish. In the present case it may simply reflect a shift to increasingly aerial routes of gas exchange at increased acclimation temperature which would be characterized by elevated $[HCO_3^-]$.

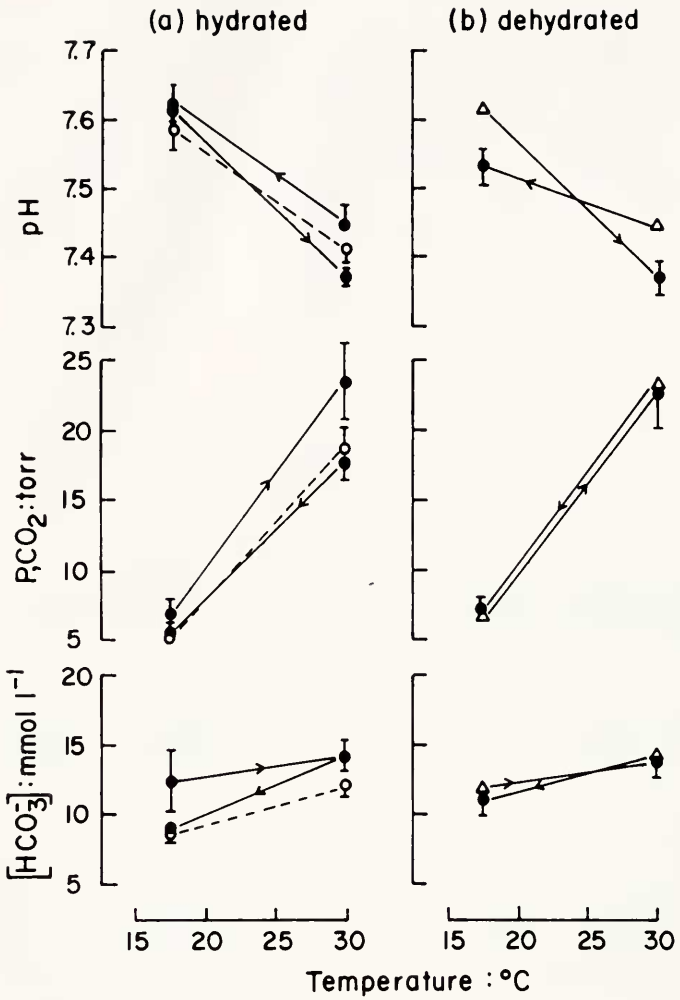

FIGURE 9. Variation in hemolymph pH, P_tCO_2, and $[HCO_3^-]$ with temperature in *Coenobita clypeatus* in the presence (a) and absence (b) of free water. Paired sample means and SE are joined together with an arrow indicating the direction in which the temperature change was made. Open symbols are values for rehydrated animals (unpaired). In (b) values are compared with corresponding control group (*i.e.*, hydrated animals at original acclimation temperature—designated by triangular symbols).

This is certainly true of other bimodal breathers such as shore crabs (Taylor and Wheatly, 1979) and adult salamanders (Burggren and Wood, 1981). The increase in HCO_3^- may compensate for an overshoot in P_tCO_2 which, otherwise occurring, would double the pH disturbance. Figure 10 illustrates this point indicating the contribution of different variables to the observed change in extracellular pH (calculated by changing one variable in the Henderson-Hasselbalch equation).

The $[HCO_3^-]$ which accumulates in the hemolymph during a temperature increase could originate from metabolic CO_2 or another fluid compartment *e.g.*, intra- to extracellular transfer (Heisler, 1978). In this respect exchange of Cl^- for endogenous bicarbonate could be one mechanism involved. Alternatively, HCO_3^- may be mobilized

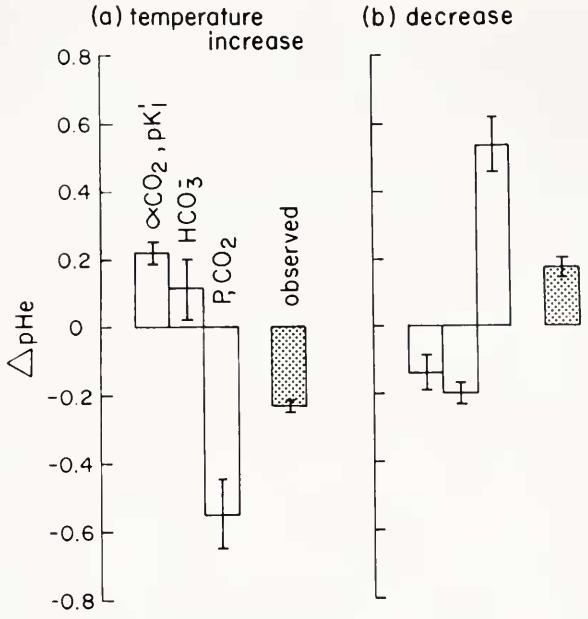

FIGURE 10. Contribution of changes in individual parameters to the relationship between pH and temperature found experimentally (stippled) in *Coenobita clypeatus* on (a) increase and (b) decrease in acclimation temperature. The label indicates the parameter which was varied.

from exoskeletal $CaCO_3$ since a disproportionate increase in hemolymph Ca^{2+} was detected (Table III). If this is so, it constitutes further evidence of "shell buffering" of hypercapnic acidosis in terrestrial species (see deFur *et al.*, 1980; Henry *et al.*, 1981; Wood and Randall, 1981) although one would expect a Ca^{2+}:base excess of 1:1 if $CaCO_3$ is the source of the buffering (Henry *et al.*, 1981).

De- and rehydration at constant temperature

Since the 14% reduction in total mass suffered by *Coenobita* included body and shell water, comparison with dehydration tolerance of other species is difficult. Nonetheless the efficacy of the adopted shell in reducing evaporation becomes apparent when comparing *settled* rates of water loss. Values of 0.7 and 1.4% initial mass day^{-1} at 18 and 30°C are a fraction of levels in both aquatic ($\simeq$12% BW day^{-1}—Herreid, 1969) and terrestrial species (*e.g.*, 5.5% in *Gecarcinus*—Bliss, 1966; 15.1% in *Ocypode*—Lutz, 1969). When deprived of water, *Coenobita* remained continuously retracted into the shell, the major chela and flattened ambulatories serving ostensibly as a reasonably water-tight operculum (see Harms, 1929; Magnus, 1960). 12% loss in body weight is generally lethal in aquatic brachyurans (Dandy and Ewer, 1961), extending upwards of 25% in inland species such as *Holthuisana* which has an exceptional ability to withstand desiccation (Greenaway and Macmillen, 1978). Interruptions to the exponential loss of water (as in Fig. 2) may arise from shell water spillage occasioned by locomotion or voluntary release due to build up of toxic wastes (A. W. Pinder and B. R. McMahon, unpub.). Loss of shell water is apparent when crabs are kept on dry sand.

The exponential decrease in rate of water loss with mass (Fig. 3) parallels other studies (Schmidt-Nielsen, 1964; Herreid, 1969) implicating scale functions; however

the slope presently reported exceeds those relating to surface area:volume (−0.33) or metabolism (−0.15; Scholander *et al.*, 1953). Water loss in the hermit crab may therefore be a complex function of the surface area of the shell water to air interface. Rate of evaporative loss did not correlate with any dimension of the molluscan shell nor shell/animal association index (see Wheatly, 1984) and therefore refutes the idea of an optimal "shell fit" although we think this possibility should be pursued.

During dehydration the increase in hemolymph osmolality (Fig. 4a) suggests that some water was lost from the extracellular compartment. Disproportionate increases in hemolymph electrolyte concentrations indicated active ionoregulation with other fluid compartments at this time. Paradoxically, in the earlier study on *C. brevimanus,* (Burggren and McMahon, 1981), ion levels were maintained despite a marked increase in osmolality which presumably involved organic osmolytes such as free amino acids increasing in response to the hyperosmotic condition developing in the blood.

The hemolymph acidosis during dehydration was possibly attributable to a shift in the ratio of cations to anions (Table IVa; Fig. 7). However the acid-base disturbance resulting from respiratory causes was greater than previously reported (Burggren and McMahon, 1981). It remains to be determined whether the increase in P,CO_2 relates to loss of an aquatic route for CO_2 excretion as shell water evaporates or to hypoventilation in order to minimize water lost in the respiratory stream (Taylor and Wheatly, 1981). The parallel disappearance of HCO_3^- and Ca^{2+} from the hemolymph during dehydration (Table IVa) suggests deposition of $CaCO_3$. In the absence of an external source of HCO_3^-, the CO_3^{2-} store could have originated from retention of respiratory CO_2 (see Wood and Randall, 1981).

The reappearance of lactate on rehydration (Table IVb) would suggest that it was sequestered intracellularly during dehydration and a similar conclusion can be made for K^+. On the whole, the ionic events occurring on rehydration were more uniform (Fig. IVb; *c.f.* IVa), suggesting a more passive physiological response.

Temperature acclimation in dehydrating crabs

Although total water loss was identical in all dehydration treatments, animals simultaneously undergoing a change in acclimation temperature exhibited less pronounced changes in circulating ion levels (Fig. 5). This could be explained if blood volume was maintained at the increased expense of shell and tissue fluid, although we have no evidence to support this. Lutz (1969) observed a similar strategy under severe dehydration (≃25% BW) in *Sudanonautes.* Furthermore temperature may alter the distribution of body water. Since Cl^- exhibited a disproportionately large increase in response to the combined stimulus (Fig. 5), one would expect this anion excess to reduce the strong ion difference (Stewart, 1978—SID calculated as $[Na^+] + [K^+] + [Ca^{2+}] + [Mg^{2+}] - [Cl^-] - [HCO_3^-]$). Yet a significant acidosis only occurred on warming, from which one can assume that ions not presently measured contributed to the SID.

Coenobita regulated hemolymph pH in the face of temperature change by ventilatory control of P,CO_2 irrespective of its relative state of hydration (Fig. 9). Truchot (1973) similarly found that ventilatory control mechanisms were predominant in the intertidal shore crab in both air and water. An increase in acclimation temperature, however, did lessen the acid-base disturbance resulting from dehydration (Table V; Fig. 6) which has ecophysiological relevance since these two parameters will frequently change together in the environment. The reverse situation, *i.e.,* dehydration combined with a reduction in temperature, would rarely occur in nature unless animals were washed ashore by warm currents in subtropical areas (de Wilde, 1973); it is not surprising that the stimuli show no physiological interaction.

In summary, the present investigation suggests that loss to circulating volume in dehydrated hermit crabs is minimized by simultaneous exposure to temperature variation which, in poikilotherms, elicits a number of physiological responses. This attempt to regulate the composition of extracellular fluid exemplifies hierarchical homeostatic regulation in response to combined environmental stimuli. Although a reservoir of water is conveyed by this species into the aerial environment, it is not essential for pH regulation during temperature variation, which continues in a fashion characteristic of other air-breathers. The physiological significance of the shell water remains as yet undetermined.

ACKNOWLEDGMENTS

We should like to thank Alan Pinder for allowing us access to unpublished data. The investigation was funded by NSERC grant A 5762 to B.R.M. AHFMR (PDF to M.G.W.) and NSF (grant PCM 80-03752 to W.W.B.) are also gratefully acknowledged for financial support.

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